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by Rebecca J. Shaw, Simon T. Abrams, James Austin, Joseph M. Taylor, Steven Lane, Tina Dutt, Colin Downey, Min Du, Lance Turtle, J. Kenneth Baillie , Peter J.M. Openshaw, Guozheng Wang, Malcolm G. Semple, and Cheng-Hock Toh.  
(Collaborative Groups: The ISARIC4C Investigators)

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# **Circulating histones play a central role in COVID-19-associated coagulopathy and mortality**

**Short title: Circulating histones in severe COVID-19**

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**Data availability:** This work uses data provided by patients and collected by the NHS as part of their care and support #DataSavesLives. The CO-CIN data was collated by ISARIC4C Investigators. ISARIC4C welcomes applications for data and material access through our Independent Data and Material Access Committee (<https://isaric4c.net>).

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COVID-19 has highlighted the lethal consequences of immunothrombosis; i.e. the cross-talk between coagulation, inflammation and the innate immune system. These patients have significant immune cell death<sup>1</sup>, which can release pro-coagulant<sup>2</sup> and cytotoxic<sup>3</sup> histones. Histones are small positively charged proteins, typically found within the cell nucleus, which bind to negatively charged DNA. We hypothesize that circulating histones play a central role in critically ill COVID-19 patients. This translational study demonstrates that admission

histone levels were significantly elevated with increasing severity of COVID-19 infection (Mild, median=2.6µg/ml [IQR=0.7-7.6], Moderate, 10.5µg/ml [3.5-27.2], Critical, 20.0µg/ml [6.2-33.0], Non-survivors, 29.6µg/ml [11.2-60.0];  $P<.001$ ). Circulating histones associated with severe coagulopathy, inflammation and organ injury markers, including cardiac troponin. Extracellular histone levels on admission are associated with poor outcomes and independently predict 28-day mortality of hospitalised COVID-19 patients. This is the first report to indicate that circulating histones, released following immune cell death, may play a central pathological role in severe SARS-CoV-2 infection.

COVID-19 was the cause of more than 2 million deaths worldwide by February 2021<sup>4</sup>, resulting from respiratory and multi-organ failure<sup>5</sup>, with evidence of pulmonary thrombosis at post-mortem<sup>6</sup>. These patients have extensive immune cell death<sup>1</sup>, a strong acute-phase inflammatory response and coagulopathy, as well as cardiac injury<sup>1, 5</sup>. Cell death can release histones, and extracellular histones are cytotoxic, pro-inflammatory<sup>7</sup> and pro-coagulant<sup>2</sup>, leading to pulmonary thrombosis<sup>8</sup>. Extracellular histones also trigger interleukin-6 (IL-6) release to induce acute phase response, including elevation of C-reactive protein (CRP), which in turn reduces histone toxicity<sup>9</sup>. High levels of circulating histones initiate an alternative coagulation pathway during sepsis<sup>2</sup>, mediate multiple organ injury<sup>3</sup> and correlate with adverse clinical outcomes, including death<sup>10</sup>. We therefore hypothesized that high levels of histones are present in severe SARS-CoV-2 infection, and act as major mediators of coagulopathy and mortality in COVID-19 disease.

In this study, adult COVID-19 patients (n=113) were recruited at the Royal Liverpool University Hospital from 30<sup>th</sup> March 2020 to 16<sup>th</sup> May 2020, using the ISARIC WHO Clinical Characterisation Protocol for Severe Emerging Infections in the UK. Inclusion criteria were: (1) swab positive or high likelihood of infection OR (2)  $\geq 1$  of the following symptoms: fever  $\geq 38^{\circ}\text{C}$ , new cough, dyspnoea OR tachypnoea AND admitted to a healthcare

facility<sup>11</sup>. Patients were categorised into four groups: 1) Mild (minor respiratory symptoms to exclude shortness of breath OR incidental finding, where the patient required admission to hospital for reasons other than COVID-19 [such as for frailty] and was otherwise asymptomatic of COVID-19), 2) Moderate (dyspnoea, i.e. patient symptomatic with shortness of breath OR hypoxia, defined by oxygen saturations on pulse oximeter of  $\leq 93\%$  or requiring supplementary oxygen to maintain oxygen saturations  $\geq 96\%$ ), 3) Critical disease (respiratory failure requiring the administration of continuous positive airway pressure [CPAP] to maintain oxygen saturations  $\geq 96\%$  or invasive ventilation in a critical care setting) and 4) Non-survivors (died within 28 days of hospital admission). Circulating histones were quantified in patient plasma on admission (as described previously<sup>8, 12</sup>) and associations with severity of infection, coagulation, inflammatory and organ injury markers were analysed. Severity of infection was determined by the patient's most severe clinical state throughout the hospital admission according to the previously described definitions. Cytokines were measured using a Luminex-based bead array as per manufacturer's instructions [Thermo-Fisher Scientific]. Outcome measures included ventilator support days, length of hospital stay, and 28-day mortality. Ethical approval was given by the South Central - Oxford C Research Ethics Committee in England (Ref 13/SC/0149), the Scotland A Research Ethics Committee (Ref 20/SS/0028), and the WHO Ethics Review Committee (RPC571 and RPC572, 25 April 2013). Local approval was granted by the North West - Haydock Research Ethics Committee (REC reference 20/NW/0332).

The Kruskal-Wallis test was used for comparison of continuous variables, presented as median [interquartile range; IQR]; the Fishers Exact/Chi squared test for comparison of categorical variables, presented as counts [percentage]. Circulating histone levels were measured by Western Blot, using purified histone as the standard, and analysed either as continuous variables or categorised based on a previously determined threshold for

cytotoxicity (30µg/mL)<sup>3, 7</sup>. Mann-Whitney U test was used to compare categorical histone levels to continuous clinical variables. Correlation analysis was performed using Spearman's rank. Receiver Operating Characteristic (ROC) curve analysis and multivariate regression (adjusted for age, gender, ethnicity and co-morbidities) assessed admission histone levels in predicting 28-day mortality. Kaplan-Meier survival curve analysis was performed to analyse the probability of mortality over time. Statistical tests were performed on SPSS (IBM, version 25). A 2-tailed P value of <.05 was considered significant.

One hundred and thirteen COVID-19 patients were studied (Table 1): median age 65.0 years [IQR=51.0-78.0 years], 65 patients were male [57.5%], 96 of white ethnicity [85.0%]. Disease severity was associated with coagulation activation (Table 1), characterised by elevated D-dimer (P=.017) and prolonged prothrombin time (P=.005), and a pro-inflammatory phenotype characterised by elevated CRP (P<.001) and IL-6 (P=.002) on hospital admission, as well as with hypoxia and cardiac injury (Table 1). Median hospital length of stay was 10 days [IQR, 3-20 days] and 25 patients [22.1%] died within 28 days.

Circulating histone levels on admission were significantly elevated in COVID-19 patients compared to normal controls and were associated with increasing severity of infection (Figure 1A and B; Healthy controls, median=2.9µg/ml [IQR=1.5-3.3]; Mild, 2.6µg/ml [0.7-7.6]; Moderate, 10.5µg/ml [3.5-27.2]; Critical, 20.0µg/ml [6.2-33.0]; Non-survivors, 29.6µg/ml [11.2-60.0]; P<.001). Circulating histone levels strongly correlated with D-dimer levels (R=.606), indicating potential involvement of extracellular histones in COVID-19 coagulopathy. Positive association with organ injury markers, including bilirubin (R=.531), creatinine (R=.501) and cardiac troponin (R=.486), indicates the possible role of histone-induced cytotoxicity in multiple organ injury. Strong associations with fibrinogen (R=.632), CRP (R=.735) and IL-6 (R=.677) confirmed histone-initiated acute phase response<sup>9</sup>. Negative



correlation with lymphocyte count ( $R=-.446$ ) suggests that lymphocyte and other immune cell death might be a major source of circulating histones in COVID-19 infection.

Using a  $30\mu\text{g/ml}$  cytotoxic histone threshold<sup>3, 7</sup>, patients over the threshold ( $n=29$ ) had significantly higher D-dimer ( $2267.0\text{ng/ml}$  [ $1227.0-5235.0$ ] vs  $1128.0\text{ng/ml}$  [ $589.0-1844.3$ ],  $P=.001$ ), fibrinogen ( $6.6\text{g/L}$  [ $4.6-7.6$ ] vs  $4.8\text{g/L}$  [ $3.9-5.7$ ],  $P=.012$ ), IL-6 ( $226.2\text{pg/ml}$  [ $90.6-518.9$ ] vs  $71.8\text{pg/ml}$  [ $35.2-111.4$ ],  $P<.001$ ) and CRP levels ( $186\text{mg/L}$  [ $108.5-247.5$ ] vs  $48.0\text{mg/L}$  [ $10.0-107.5$ ],  $P<.001$ ) than those patients below the threshold (Table 2). These patients also had significantly reduced  $\text{SpO}_2$  than those with circulating histones  $<30\mu\text{g/ml}$  (oxygen saturations  $92.0\%$  [ $85.8-94.0$ ] vs  $95.0\%$  [ $93.5-97.0$ ],  $P=.001$ ), required critical care admission ( $P<.001$ ), with longer duration of mechanical ventilation ( $R=.635$ ) and hospital stay ( $R=.654$ ).

Circulating histone levels were significantly higher in non-survivors than those who survived ( $29.6\mu\text{g/ml}$  [ $11.2-60.0$ ] vs  $8.6\mu\text{g/ml}$  [ $3.1-24.8$ ],  $P=.002$ ), and accordingly, patients with histones  $>30\mu\text{g/ml}$  were more likely to die ( $13/29$  [ $44.8\%$ ] vs  $12/84$  [ $14.3\%$ ],  $P=.001$ ). Patients who died were significantly older than those who survived (Table 2,  $76$  years [ $66-86$ ] vs  $59$  years [ $46-72$ ]  $P<.001$ ). Compared to survivors, non-survivors had evidence of consumptive coagulopathy with lower platelet counts ( $P=.003$ ), prolonged prothrombin time ( $P=.028$ ), elevated D-dimer ( $P=.017$ ) and reduced antithrombin levels ( $P=.048$ ). Furthermore, in non-survivors, lymphocyte counts ( $P=.001$ ), and oxygen saturations ( $P=.005$ ) were significantly reduced, and IL-6 ( $P=.021$ ), CRP ( $P=.013$ ), troponin ( $P<.001$ ), bilirubin ( $P=.041$ ) and creatinine ( $P=.024$ ) were elevated when compared to survivors (Table 2).

Univariate analysis using continuous circulating histones demonstrated that rising histone levels were associated with mortality (odds ratio  $=1.031$  (95% CI= $1.013-1.049$ ,  $P=.001$ ). Using categorical data where patients were stratified based on  $\geq 30\mu\text{g/ml}$  threshold<sup>3, 7</sup>, similar

results were obtained (Figure 1C, OR=4.875 (95% CI=1.879-12.649, P=.001), demonstrating that patients with high circulating histone levels on admission had higher risks of mortality. Subsequent multivariate analysis demonstrated that histones were independently associated with mortality after adjustment for age, gender, ethnicity and co-morbidity, when histone levels were treated as either continuous (odds ratio=1.032; 95% CI=1.013-1.051, P=.001) or categorical variables (odds ratio=5.404; 95% CI=1.852-15.770, P=.002). ROC curve analysis shows an area under the curve [AUC] of .708 (95% CI=.589-.827, P=.002). Kaplan-Meier survival curve demonstrated a significant increase in the probability of mortality during 28-days in patients with histones  $\geq 30\mu\text{g/ml}$  (Figure 1D, P=.001).

Coagulopathy has emerged as a key feature of severe COVID-19 and has been linked to increased mortality<sup>13</sup>. It has been documented that extracellular histones, released following cell death, are drivers of coagulation by activating platelets<sup>7</sup>, generating thrombin<sup>2</sup> and damaging endothelial cells<sup>8</sup> to induce coagulopathy in critical illness<sup>3</sup>. This is the first report to demonstrate high levels of circulating histones in SARS-CoV-2 infection, with levels strongly associated with coagulopathy, suggesting their involvement in thrombosis in severe cases<sup>14</sup>.

High levels of circulating histones reflect the extent of cellular death, such as lymphopenia or NETosis<sup>15</sup>, which may be a major source of circulating histones in COVID-19. Histone release following cell death triggers IL-6 release to induce the acute-phase response<sup>8</sup>. We found that circulating histone levels significantly correlated with IL-6 and acute-phase protein levels, including fibrinogen and CRP, indicating histone-induced acute phase response in patients with COVID-19.

Extracellular histones disrupt cell membranes through phospholipid binding to induce cytotoxic effects on cells, including endothelial cells<sup>8</sup> and cardiomyocytes<sup>12</sup>. This study

demonstrates circulating histones associated with cardiac injury, which is frequently seen with severe COVID-19, and associated with poor outcomes<sup>5</sup>. Therefore, the cytotoxic and pro-coagulant properties of circulating histones may be an underlying molecular mechanism contributing to disease severity and poor outcomes (Figure 1E).

In conclusion, this is the first report to quantify high levels of circulating histones in viral infection and demonstrate that extracellular histones play a central role in the development of immunothrombosis and critical illness in COVID-19.

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	Total	Mild	Moderate	Critical	Non-survivors	P value <sup>a</sup>
Total number (n)	113	30	38	20	25	..
<b>Demographics &amp; Comorbidities</b>						
Age (years), Median [IQR]	65.0 [51.0, 78.0]	63.5 [42.0, 70.0]	67.0 [57.5, 81.5]	51.0 [42.8, 54.5] <sup>*,¥</sup>	76.0 [66.0, 86.0] <sup>*,†</sup>	<.001
Male, No. [%]	65 [57.5]	15 [50.0]	20 [52.6]	14 [70.0]	16 [64.0]	.428
White ethnicity, No. [%]	96 [85.0]	26 [86.7]	35 [92.1]	11 [55.0]	24 [96.0]	.001
Smoking history, No. [%]	38 [33.6]	10 [33.3]	16 [42.1]	4 [20.0]	8 [32.0]	.033
Hypertension, No. [%]	36 [31.9]	8 [26.7]	12 [31.6]	5 [25.0]	11 [44.0]	.474
Asthma/COPD, No. [%]	29 [25.7]	14 [46.7]	10 [26.3]	1 [5.0]	4 [16.0]	.005
Diabetes mellitus, No. [%]	29 [25.7]	5 [16.7]	10 [26.3]	5 [25.0]	9 [36.0]	.443
Ischaemic heart disease, No. [%]	16 [14.2]	3 [10.0]	8 [21.1]	0 [0.0]	5 [20.0]	.116
Chronic kidney disease, No. [%]	15 [13.3]	3 [10.0]	10 [26.3]	0 [0.0]	2 [8.0]	.025
Histones (µg/ml), Median [IQR]	10.8 [3.2, 29.9]	2.6 [0.7, 7.6]	10.5 [3.5, 27.2] *	20.0 [6.2, 33.0] *	29.6 [11.2, 60.0] <sup>*,¥</sup>	<.001
<b>Peripheral blood cell counts</b>						
White blood cells (x10 <sup>9</sup> /L), Median [IQR]	8.5 [5.8, 11.8]	8.2 [6.6, 10.7]	9.8 [5.9, 12.3]	8.1 [6.5, 10.8]	8.1 [5.2, 11.3]	.623
Neutrophils (x10 <sup>9</sup> /L), Median [IQR]	6.4 [4.0, 9.3]	5.9 [3.8, 8.0]	7.0 [4.1, 9.8]	6.4 [4.0, 9.0]	7.2 [4.0, 11.2]	.748
Lymphocytes (x10 <sup>9</sup> /L), Median [IQR]	1.0 [0.7, 1.6]	1.2 [0.8, 1.7]	1.1 [0.8, 1.4]	1.1 [0.9, 2.1]	0.7 [0.4, 1.1] <sup>*,¥,†</sup>	.009
Haemoglobin (g/L), Median [IQR]	129.0 [117.8, 145.3]	126.0 [119.0, 145.0]	123.0 [113.8, 139.8]	134.5 [131.0, 146.0] <sup>¥</sup>	136.0 [107.0, 147.0]	.122
Platelets (x10 <sup>9</sup> /L), Median [IQR]	236.5 [170.3, 296.0]	253.0 [177.0, 311.0]	243.5 [113.8, 139.8]	250.5 [207.3, 299.3]	174.0 [124.0, 250.0] <sup>*,¥,†</sup>	.026
<b>Coagulation parameters</b>						
PT (seconds), Median [IQR]	13.2 [12.1, 14.4]	12.1 [11.2, 13.0]	13.1 [12.1, 14.4] *	13.4 [13.1, 14.2] *	14.1 [12.4, 20.7] *	.005
aPTT (seconds), Median [IQR]	30.6 [28.2, 33.6]	31.0 [28.9, 32.7]	30.5 [28.3, 32.6]	32.0 [29.1, 33.7]	30.0 [28.2, 37.6]	.775
Fibrinogen (g/L), Median [IQR]	4.8 [3.9, 6.5]	4.2 [2.8, 5.4] <sup>†</sup>	4.8 [4.4, 6.7]	6.5 [5.4, 6.6] *	4.5 [3.1, 4.9] <sup>†</sup>	.010
D-dimer (ng/ml), Median [IQR]	1227.0 [687.0, 2141.5]	755.5 [431.5, 1744.0]	1315.0 [832.5, 2176.3] *	950.0 [602.0, 1728.0]	1630.0 [1117.0, 4334.0] <sup>*,†</sup>	.017
Antithrombin (%), Median [IQR]	80.0 [61.0, 100.0]	81.0 [57.5, 98.5]	80.0 [61.5, 97.5]	98.0 [80.3, 114.8] <sup>*,¥</sup>	70.0 [59.0, 87.0] <sup>†</sup>	.024
<b>Pro-inflammatory markers</b>						
IL-6 (pg/ml), Median [IQR]	79.0 [40.5, 131.9]	53.2 [15.0, 83.1]	70.5 [41.9, 115.0]	166.7 [75.6, 214.7] *	107.7 [81.3, 269.8] <sup>*,¥</sup>	.002
C-reactive protein (mg/L), Median [IQR]	61.0 [21.0, 153.5]	16.0 [3.5, 53.8]	52.0 [23.3, 146.3] *	145.0 [97.0, 202.5] <sup>*,¥</sup>	105.0 [71.0, 192.0] <sup>*,¥</sup>	<.001
<b>Organ injury markers</b>						
Troponin T (ng/L), Median [IQR]	12.0 [5.0, 35.0]	8.0 [5.0, 16.0]	16.0 [6.8, 47.3] *	6.5 [5.0, 10.5] <sup>¥</sup>	35.0 [17.0, 58.0] <sup>*,†</sup>	<.001
Bilirubin (µmol/L), Median [IQR]	9.0 [6.0, 14.0]	8.0 [4.5, 13.0]	8.0 [6.0, 15.0]	9.0 [6.0, 12.5]	12.0 [8.0, 16.5] *	.142
ALT (U/L), Median [IQR]	25.5 [14.5, 45.0]	21.0 [11.5, 55.0]	19.0 [11.5, 38.0]	33.5 [29.0, 59.5] <sup>¥</sup>	28.5 [15.8, 44.3]	.163
Creatinine (µmol/L), Median [IQR]	77.0 [63.0, 105.0]	74.5 [62.0, 82.3]	78.0 [60.8, 104.3]	80.0 [57.8, 96.0]	102.0 [71.0, 180.0] *	.125
SpO2 (%), Median [IQR]	95.0 [92.0, 97.0]	97.0 [95.0, 98.0]	94.5 [92, 96] *	94.0 [92.0, 96.5] *	92.0 [78.5, 96.0] *	<.001
<b>Outcomes</b>						
Length of stay (days), Median [IQR]	10.0 [3.0, 20.0]	2.0 [1.0, 13.8]	10.0 [6.0, 22.0] *	17.0 [9.5, 43.8] *	..	<.001
Ventilator support (days), Median [IQR]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	2.0 [0.0, 9.3]	0.0 [0.0, 8.0]	<.001

**Table 1. Demographics, peripheral blood measurements and outcomes for disease severity groups in COVID-19 infection.** <sup>a</sup> P value for comparisons mild vs moderate vs critical disease vs non-survivors collectively. Performed using Kruskal-Wallis test. \* Significant vs mild disease, ¥ Significant vs moderate disease, † Significant vs critical disease.

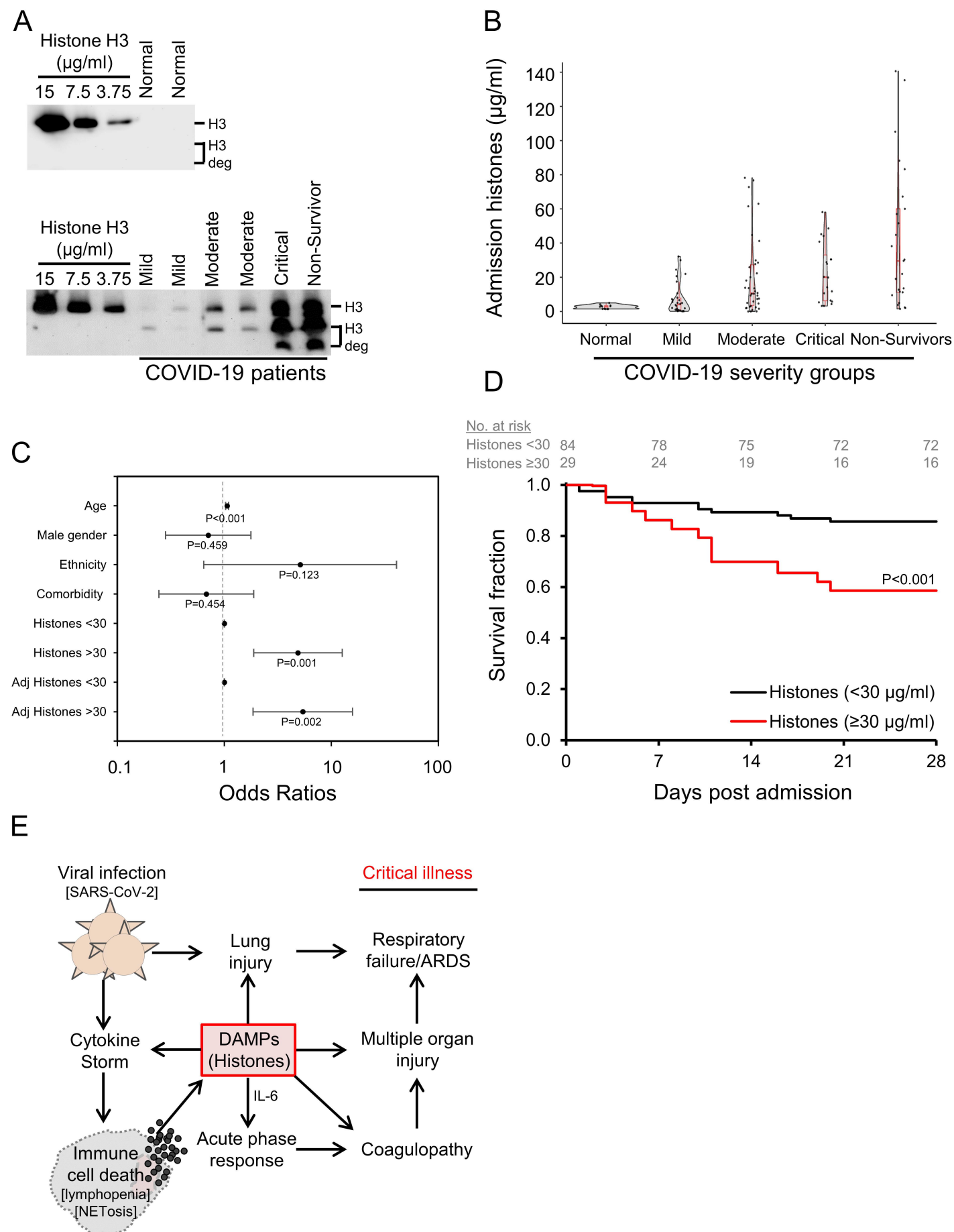
	Survivors	Non-survivors	P value <sup>a</sup>	Histones <30µg/ml	Histones ≥30µg/ml	P value <sup>b</sup>
Total number (n)	88	25	..	84	29	..
<b>Demographics &amp; Comorbidities</b>						
Age (years), Median [IQR]	59.0 [45.8, 72.3]	76.0 [66.0, 86.0]	<.001	63.0 [47.8, 76.0]	66.0 [57.0, 80.0]	.224
Male, No. [%]	49 [55.7]	16 [64.0]	.458	48 [57.1]	17 [58.6]	.890
White ethnicity, No. [%]	72 [81.8]	24 [96.0]	.113	73 [86.9]	23 [79.3]	.324
Smoking history, No. [%]	30 [34.1]	8 [32.0]	.845	28 [33.3]	10 [34.5]	.910
Hypertension, No. [%]	25 [28.4]	11 [44.0]	.140	28 [33.3]	8 [27.6]	.567
Asthma/COPD, No. [%]	25 [28.4]	4 [16.0]	.301	25 [29.8]	4 [13.8]	.138
Diabetes mellitus, No. [%]	20 [22.7]	9 [36.0]	.180	21 [25.0]	8 [27.6]	.783
Ischaemic heart disease, No. [%]	11 [12.5]	5 [20.0]	.343	13 [15.5]	3 [10.3]	.758
Chronic kidney disease, No. [%]	13 [14.8]	2 [8.0]	.515	11 [13.1]	4 [13.8]	>.999
Histones (µg/ml), Median [IQR]	8.6 [3.1, 24.8]	29.6 [11.2, 60.0]	.002	6.1 [2.0, 13.5]	51.6 [38.2, 72.8]	<.001
<b>Peripheral blood cell counts</b>						
White blood cells (x10 <sup>9</sup> /L), Median [IQR]	8.7 [6.1, 11.8]	8.1 [5.2, 11.3]	.387	8.0 [5.7, 11.0]	9.8 [6.7, 13.3]	.084
Neutrophils (x10 <sup>9</sup> /L), Median [IQR]	6.2 [4.0, 8.9]	7.2 [4.0, 11.2]	.563	5.7 [3.6, 8.2]	9.1 [6.1, 12.2]	.001
Lymphocytes (x10 <sup>9</sup> /L), Median [IQR]	1.1 [0.8, 1.7]	0.7 [0.4, 1.1]	.001	1.2 [0.8, 1.7]	0.8 [0.5, 1.1]	.007
Haemoglobin (g/L), Median [IQR]	128.0 [118.0, 144.0]	136.0 [107.0, 147.0]	.740	128.0 [118.0, 145.0]	131.0 [116.0, 147.0]	.740
Platelets (x10 <sup>9</sup> /L), Median [IQR]	248.0 [181.0, 299.0]	174.0 [124.0, 250.0]	.003	237.5 [174.3, 295.8]	215.0 [155.8, 296.8]	.410
<b>Coagulation parameters</b>						
PT (seconds), Median [IQR]	13.0 [11.8, 14.1]	14.1 [12.4, 20.7]	.028	12.8 [11.8, 14.0]	13.8 [13.3, 15.6]	.005
aPTT (seconds), Median [IQR]	30.9 [28.4, 32.9]	30.0 [28.2, 37.6]	.858	30.7 [28.7, 34.0]	29.5 [28.0, 32.6]	.268
Fibrinogen (g/L), Median [IQR]	5.3 [4.1, 6.5]	4.5 [3.1, 4.9]	.091	4.7 [3.9, 5.7]	6.6 [4.6, 7.6]	.012
D-dimer (ng/ml), Median [IQR]	1166.0 [619.0, 2038.0]	1630.0 [1117.0, 4334.0]	.017	1128.0 [589.0, 1844.3]	2267.0 [1227.0, 5235.0]	.001
Antithrombin (%), Median [IQR]	83.0 [62.5, 102.5]	69.5 [55.8, 81]	.048	82.0 [59.0, 100.4]	77.0 [69.0, 99.0]	.971
<b>Pro-inflammatory markers</b>						
IL-6 (pg/ml), Median [IQR]	73.9 [36.6, 125.4]	107.7 [81.3, 269.8]	.021	71.8 [35.2, 111.4]	226.2 [90.6, 518.9]	<.001
C-reactive protein (mg/L), Median [IQR]	50.0 [15.3, 149.0]	105.0 [71.0, 192.0]	.013	48.0 [10.0, 107.5]	186.0 [108.5, 247.5]	<.001
<b>Organ injury markers</b>						
Troponin T (ng/L), Median [IQR]	5.0 [10.0, 23.0]	35.0 [17.0, 58.0]	<.001	10.0 [5.0, 24.0]	25.0 [9.8, 57.3]	.011
Bilirubin (µmol/L), Median [IQR]	8.0 [5.0, 13.0]	12.0 [8.0, 16.5]	.041	8.0 [5.0, 13.0]	11.0 [8.5, 16.3]	.016
ALT (U/L), Median [IQR]	25.0 [12.8, 45.0]	28.5 [15.8, 44.3]	.727	20.5 [12.8, 38.3]	36.5 [25.5, 55.3]	.062
Creatinine (µmol/L), Median [IQR]	76.0 [61.0, 96.8]	102.0 [71.0, 180.0]	.024	76.0 [62.5, 99.3]	96.0 [65.0, 154.0]	.127
SpO2 (%), Median [IQR]	95.0 [93.0, 97.0]	92.0 [78.5, 96.0]	.005	95.0 [93.5, 97.0]	92.0 [85.8, 94.0]	.001
<b>Outcomes</b>						
Length of stay (days), Median [IQR]	10.0 [3.0, 20.0]	..	..	8.0 [2.5, 15.5]	28.0 [13.0, 41.5]	<.001
Ventilator support (days), Median [IQR]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	.347	0.0 [0.0, 0.0]	0.0 [0.0, 8.0]	<.001
Mortality at 28 days, No. [%]	0 [0]	25 [100]	<.001	12 [14.3]	13 [44.8]	.001

**Table 2. Demographics, peripheral blood measurements and outcomes of COVID-19 patients.** <sup>a</sup> P value for survivors vs non-survivors. <sup>b</sup> P value for toxic histone levels vs. non-toxic. Performed using Mann-Whitney U test for continuous variables and Fishers Exact/Chi squared tests for categorical variables.

## Figure legends

**Figure 1. High levels of circulating histones on hospital admission are associated with disease severity and mortality in COVID-19.** Typical western blots (A) and quantification (B) of histone levels in healthy controls (n=12), mild (n=30), moderate (n=38) and critical disease (n=20) and non-survivors (n=25) with COVID-19 infection. Circulating histone levels were higher with increasing disease severity ( $P<.001$ ). Histone levels were higher in non-survivors compared to the moderate ( $P=.023$ ), mild groups ( $P<.001$ ) and to normal healthy controls ( $P<.001$ ). Histone levels were higher in the critical group compared to mild groups ( $P<.001$ ) and normal healthy controls ( $P<.001$ ). Histone levels were higher in the moderate group compared to the mild group ( $P=.007$ ) and normal healthy controls ( $P=.002$ ). (C) Multivariate analysis of crude and adjusted odds ratios (with patients adjusted for age, gender, BAME ethnicity and comorbidities including smoking, hypertension, asthma/COPD, diabetes, ischaemic heart disease and chronic kidney disease). Circulating histone levels  $>30\mu\text{g/ml}$  were independently associated with 28-day mortality. (D) Kaplan-Meier survival curve for the probability of mortality during 28 days. Patients were stratified based on circulating histones levels on admission ( $<30\mu\text{g/ml}$  vs  $\geq 30\mu\text{g/ml}$ ). (E) Diagram to propose that circulating histones play a central pathological role in the development of severe COVID-19.

# Figure 1





## **Appendix 1**

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